Depth Distribution of *Rotylenchulus reniformis* Under Different Tillage and Crop Sequence Systems

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Accepted for publication 25 March 2003.

ABSTRACT

Westphal, A., and Smart, J. R. 2003. Depth distribution of *Rotylenchulus reniformis* under different tillage and crop sequence systems. Phytopathology 93:1182-1189.

The population density of the reniform nematode, *Rotylenchulus reniformis,* was monitored at depths of 0 to 30, 30 to 60, 60 to 90, and 90 to 120 cm in a tillage and crop sequence trial in south Texas in 2000 and 2001. Main plots were subjected to three different tillage systems: conventional tillage (moldboard plowing and disking), ridge tillage, and no-tillage. Subplots were planted with three different crop sequences: spring cotton and fall corn every year; spring cotton and fall corn in one year, followed by corn for two years; and cotton followed by corn and then grain sorghum, one spring crop per year. The population density of *R. reniformis* on corn and grain sorghum was low throughout the soil profile. In plots planted with spring cotton and fall corn every year, fewer nematodes were found at depths of 60 to 120 cm in the no-tillage and ridge tillage systems than in the conventional tillage system. Population densities were lower at depths of 0 to 60 cm than at 60 to 120 cm. Soil moisture and cotton root length did not affect nematode population densities in the field. When soil was placed in pots and planted with cotton in the greenhouse, lower population densities developed in soil taken from depths of 0 to 60 cm than in soil from depths of 60 to 120 cm. Final nematode populations were similar in size in soil from the different tillage systems, but reproductive factors were higher in soil from plots with reduced-tillage systems than in soil from plots with conventional tillage. Reduced-tillage practices lowered the risk of increases in *R. reniformis* populations and reduced population densities following 2 years of nonhosts throughout soil depths, but population densities resurged to the same high levels as in soil planted with cotton every year during one season of cotton.

Additional keywords: crop rotation, nematode depth distribution.

The reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira 1940, is distributed in subtropical and tropical regions worldwide (20). It occurs at a high rate of incidence throughout the southern United States east of New Mexico (13,32) and proliferates best at soil temperatures of 25 to 29.5°C (28). *Rotylenchulus reniformis* infects cotton (*Gossypium* spp.) (46) and a broad range of vegetable crops (33). It can cause yield losses of 30 to 40% in upland cotton, *G. hirsutum,* and 10 to 15% in Egyptian cotton, *G. barbadense* (10,26). In some areas, such as in Louisiana, infestation of 56% of the crop area has been reported (25). The loss in cotton production in 2001 due to reniform nematode damage was estimated to be 395,788 bales (approximately 8.6 × 10^7 kg) (2).

Rotylenchulus reniformis prefers fine-textured soils (32), and it was believed that its population density is greatest at depths of 0 to 15 cm (9). Recently, it was found at greater depths and in greater population densities than expected for plant-parasitic nematodes under annual crops (29). In a preliminary survey of the depth distribution of *R. reniformis* under cotton in Arkansas, Louisiana, and Texas, about half the fields had high population densities down to a depth of 100 cm, and in at least half the samples at least half of the populations of *R. reniformis* were below the typically sampled 30-cm depth (29). In a Texas study, the greatest population densities occurred at depths of 90 to 105 cm (30). A similar distribution pattern was also found under soybean susceptible to the nematode (50). Several nematicides have been found effective in controlling *R. reniformis* (3,10) in the top 30 cm of soil, but effects on deeper nematode populations may be limited.

In general, in integrated nematode management, all cultural tools and control measures, including host plant resistance, biological control, and crop rotation, are implemented to keep nematode populations below economic threshold levels (1). These methods all depend on the ecology of the nematode pest. Crop rotation has been effective in managing plant-parasitic nematodes in low-value crops (1,5,18,36). Various crop sequences for the management of *R. reniformis* have been proposed, including the most easily adopted rotation with nonhost plants, such as corn (35), grain sorghum (21), or soybean cultivars resistant to the nematode (7,11).

The production of cotton, like the production of many low-value crops, is undergoing a transition to reduced tillage or no-tillage, with economic and environmental benefits, e.g., reduced fuel costs and reduced nutrient runoff (22,44). The shift to production systems with less soil disturbance has raised concerns about effects on pathogen pressure (45).

In soybean and corn production, the influence of tillage has been found to vary according to the plant-parasitic nematode under investigation (24). In several studies, the population density of *Heterodera glycines* was lower in no-tillage plots than in conventionally tilled plots (19,47). It was uncertain why these differences in population density occurred, but it was hypothesized that biological control or a reduction in nematode feeding sites due to decreased soil aeration, resulting in host plant root death, was involved as a cause (19).

In cotton production, particularly in the lower Rio Grande Valley of Texas, the shift to conservation tillage lags that of crop production in the U.S. Midwest, although economic benefits from

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Publication no. P-2003-0623-01R
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reduced tillage in this region have been demonstrated (38,41). Reduced tillage has also been found to improve soil tilth, by increasing organic matter content under a no-tillage system compared to conventional tillage (40). *Rotylenchulus reniformis* is abundant in south Texas (43). Concerns about the effect of a shift to no-tillage, with less soil disturbance, on *R. reniformis* population densities in cotton cropping sequences prompted this study.

The objective of this project was to evaluate the impact of reduced tillage systems on population densities of *R. reniformis* under different crop sequences. For this purpose, population density was monitored in a two-factor factorial trial with tillage treatments (conventional tillage, ridge tillage, and no-tillage) and crop sequences typical of south Texas. This test has been in place at the USDA ARS North Farm, Weslaco, Texas, since 1992.

MATERIALS AND METHODS

In the present study, the population density of *R. reniformis* and soil moisture were monitored in 30-cm increments over a depth of 120 cm during 2000 and 2001. In the 2001 cotton crop, soil temperature was also monitored at the different depths, and cotton root length was measured at midseason and at harvest. In a greenhouse test, nematode population densities were determined in soils collected at the beginning of the growing season, infested with additional *R. reniformis,* and planted with cotton. Population densities developing in place in the field and in the removed soils in the greenhouse test were compared.

Field trial. A long-term two-factor factorial field trial was established at North Farm in 1992 on a silty clay loam soil (56% sand, 19% silt, and 25% clay; pH 8 in the top 10 cm). In a previous study of *R. reniformis* in this field, only the upper 20 cm was sampled, and higher population densities were found in the reduced-tillage plots (6). When soil depths were examined separately, the following textures were found: 0 to 30 cm—69% sand, 7% silt, and 24% clay; 30 to 60 cm—73% sand, 3% silt, and 24% clay; 60 to 90 cm—83% sand, 1% silt, and 16% clay; 90 to 120 cm—71% sand, 5% silt, and 16% clay. Four blocks (0.9 to 1.3 ha), 73.5 to 105.9 m long, were each divided into three main plots for the three tillage systems, and these strips were further divided into three subplots (13.5 m wide) for three different crop sequences. Main plots and subplots were randomized.

The three tillage systems were conventional tillage with a moldboard plow (CT); ridge tillage (RT), a reduced-tillage system; and a no-tillage system (NT) (preplant no-tillage from 1992 to 1995 and no-tillage after 1996). Tillage operations were conducted with standard agricultural equipment from 1992 to 1995 as described earlier (39) and modified in NT after 1996. CT plots were disked after harvest, plowed, and disked three times, and then seedbeds were prepared. CT and RT plots received two cultivations during the growing season, and NT plots received no tillage.

TABLE 1. Crop sequences (CS) in trials at Weslaco, Texas, 1999–2001[a]

Year	Season	CS1	CS2	CS3
1999	Spring	Cotton	Corn	Cotton
	Fall	Corn	…	…
2000	Spring	Cotton[b]	Cotton[b]	Corn[c]
	Fall	Corn[c]	Corn[c]	…
2001	Spring	Cotton[b]	Corn[c]	Grain sorghum[d]
	Fall	Corn[c]	…	…

[a] Spring crops were planted during February to March and harvested during July to August. Fall crops were planted in August and harvested in December and January. Nematode population densities and soil moisture were measured in 2000 and 2001. Soil temperature and cotton root lengths were measured in 2001 only.

[b] *Gossypium hirsutum* L. cv. Delta Pine 655 B/RR (Delta Pine, Scott, MS).

[c] *Zea mays* L. cv. Pioneer 3025 (Pioneer, Des Moines, IA).

[d] *Sorghum bicolor* (L.) Moench cv. Pioneer 8313 (Pioneer, Des Moines, IA).

The subplots of the tillage treatments were planted with three crop sequences: an intensive cotton sequence (CS1), a cotton sequence of medium intensity (CS2), and an extensive sequence (CS3), as described in Table 1. All crops were planted with 75-cm row spacing. Spring corn was planted on 7 February 2000 and 16 February 2001, grain sorghum on 21 February, and fall corn on 17 August. Grain sorghum was harvested on 2 July, and other spring crops were harvested on 3 July and 16 July; fall corn was harvested on 8 January. Cotton was planted on 14 March 2000 and 16 March 2001 and harvested on 17 July 2000 and 30 July 2001. Natural precipitation was amended with two furrow irrigations of spring crops. Corn, cotton, and sorghum plots were treated with glyphosate, dicamba, or atrazine before planting. Corn received terbufos and tefluthrin at half the rate specified on the product label, and limited nematode suppression due to the second material was expected. All cotton plots were treated with insecticides to control the boll weevil, *Anthonomus grandis.* Insecticidal active ingredients included methyl, oxamyl, tribufos, and azinophos-methyl. To facilitate harvest, paraquat was used to defoliate the cotton.

The project was started in spring 2000 (Table 1), when the CS1 and CS2 plots were planted with cotton, and the CS3 plots were planted with corn. In fall 2000, the CS1 and CS2 plots were planted with corn, and the CS3 plots were left fallow (Table 1). In 2001, the CS1 plots were planted with cotton, the CS2 plots with corn, and the CS3 plots with grain sorghum (Table 1). Each year, three sampling sites were established in each plot (one at each end, separated from the edge of the plot by a distance of about one-fifth of the total length of the plot, and one about at the center of the length of the plot). Soil samples were collected at the planting of the spring crop (in February or March), at the midseason of the spring crop (in May or June), and at the harvest of the spring crop (in August). Whenever practical, soil samples were collected with a tractor-mounted hydraulic soil sampler (4.3 cm in diameter) (GSTS, Giddings Machine Company, Fort Collins, CO). Alternatively, a handheld subsoil sampler (ESP or ESP+, Clements and Associates, Newton, IA) was used in midseason corn (in 2000, 2 cm in diameter to a depth of 90 cm; in 2001, 3 cm in diameter to a depth of 120 cm). Each soil core was separated into four 30-cm segments (from depths of 0 to 30, 30 to 60, 60 to 90, and 90 to 120 cm). From each subsample, 50 cm^3 of soil was processed separately for nematode extraction by the Baermann funnel method (14). Nematode population density was reported as the number of vermiform reniform nematodes per 100 cm^3 of soil. Soil moisture

TABLE 2. Analysis of variance (ANOVA) of effects of tillage, crop sequence, sampling time, and sampling depth on population density of *Rotylenchulus reniformis*[a,b]

		2000		2001	
	df	Mean square	Probability	Mean square	Probability
Tillage (Tl)	2	0.695967	0.02	0.016774	0.93
Error (a)	6	0.091277	0.02	0.230869	<0.01
Crop sequence (Cs)	2	13.376343	<0.01	14.583036	<0.01
Error (b)	18	0.190680	<0.01	0.107438	<0.01
Sampling time (St)	2	11.456515	<0.01	0.144673	0.11
Cs × St	4	4.984269	<0.01	1.216290	<0.01
Error (c)	54	0.142800	<0.01	0.062766	<0.01
Sampling depth (Sd)	3	0.893819	<0.01	0.100272	0.32
Error (d)	9	0.066560	0.06	0.074582	<0.01
Tl × Sd	6	0.279270	<0.01	0.081039	0.04
Cs × Sd	6	0.447551	<0.01	0.277442	<0.01
Pooled error (e + f)	72	0.071944	…	0.035106	…
Cs × St × Sd	12	0.067020	0.04	0.053287	0.05
Error (g)	162	0.036129	…	0.029472	…

[a] Only main effects and interactions discussed in the text and the corresponding error terms are listed in this abbreviated ANOVA table.

[b] Population density data were transformed to $\log_{10}$[(vermiform nematodes)/(100 cm^3) + 1].

was determined gravimetrically at each sampling depth in cotton plots.

In 2001, in addition to the 2000 sampling and processing procedure, the soil temperature at depths of 15, 45, 75, and 105 cm was recorded by a data logger (Optic StowAway, Onset, Pocasset, MA) every 30 min in one randomly chosen block in the CS1 plots from 21 March until 6 August. At the midseason and harvest soil samplings, cotton roots were extracted from a 150-cm^3 composite of three subsamples from each CS1 plot at each sampling depth. For extraction, the composite soil sample was washed through a 6-mm-pore-size sieve to transfer it to a 19-liter bucket, in which it was stirred for 1 min in 10 liters of water. The resulting slurry was then passed through a 425-µm-pore-size sieve nested onto a 180- and a 150-µm-pore-size sieve (a modification of the procedure described in reference 23). Roots and debris from all sieves were combined on the 150-µm-pore-size sieve and transferred to a petri plate, and cotton roots were transferred manually to a 2% formaldehyde solution. Samples were stored at 4°C until they were read with a computer-assisted root scanner (Regent Instruments, Quebec, Canada). Cotton root length was recorded in centimeters per 100 cm^3 of soil.

Greenhouse test for nematode population development. In 2001, preseason soil samples were collected from CS1 plots for each block × tillage × depth origin, mixed according to tillage system and 30-cm soil layer, and passed through a screen (aperture, 6 mm). Soils were divided into 550-cm^3 portions, amended with 40 cm^3 of silty loam soil containing 100 vermiform *R. reniformis,* and placed in 1-liter pots. The endemic reniform nematode popu-

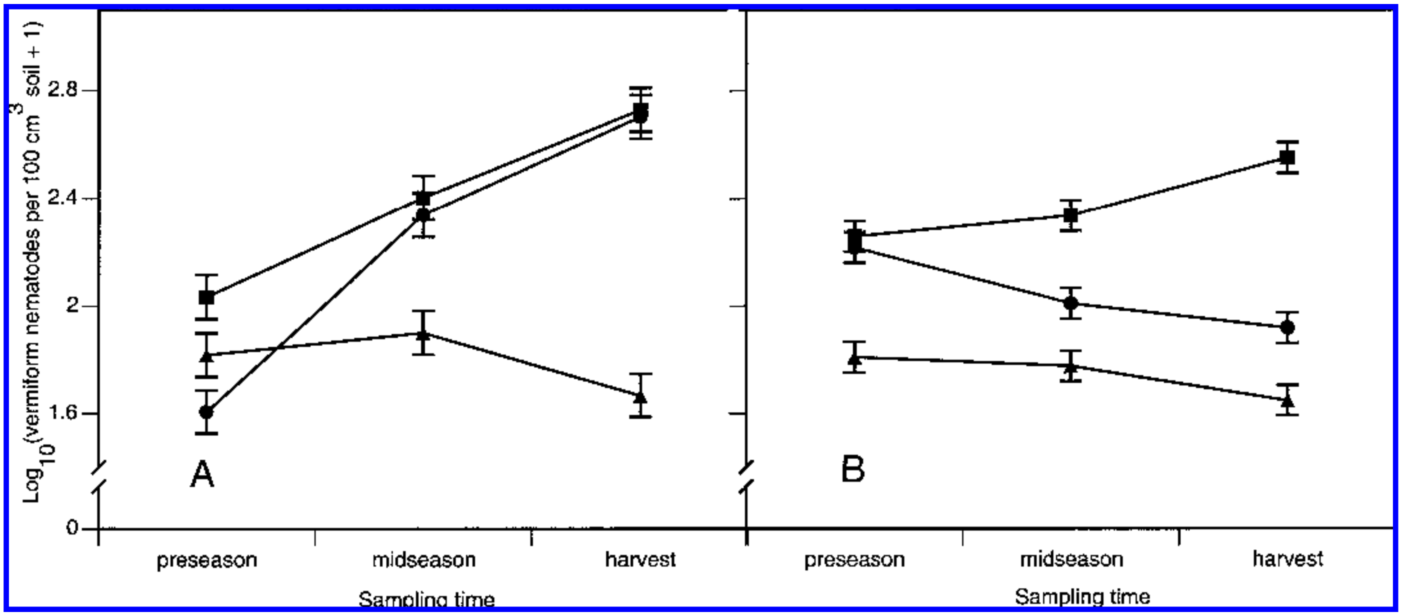

Fig. 1. Population density of *Rotylenchulus reniformis* (data transformed to $\log_{10}(x + 1)$) during the growing season, with three different crop sequences (■ = CS1; ● = CS2; ▲ = CS3), as defined in Table 1, and three tillage systems, at four sampling depths, in (**A**) 2000 and (**B**) 2001. The error bars represent the least significant interval (LSI), where LSI = 0.5 × $LSD_{0.05}$, for comparing crop sequences within a sampling time and year. Values are significantly different when the error bars do not overlap.

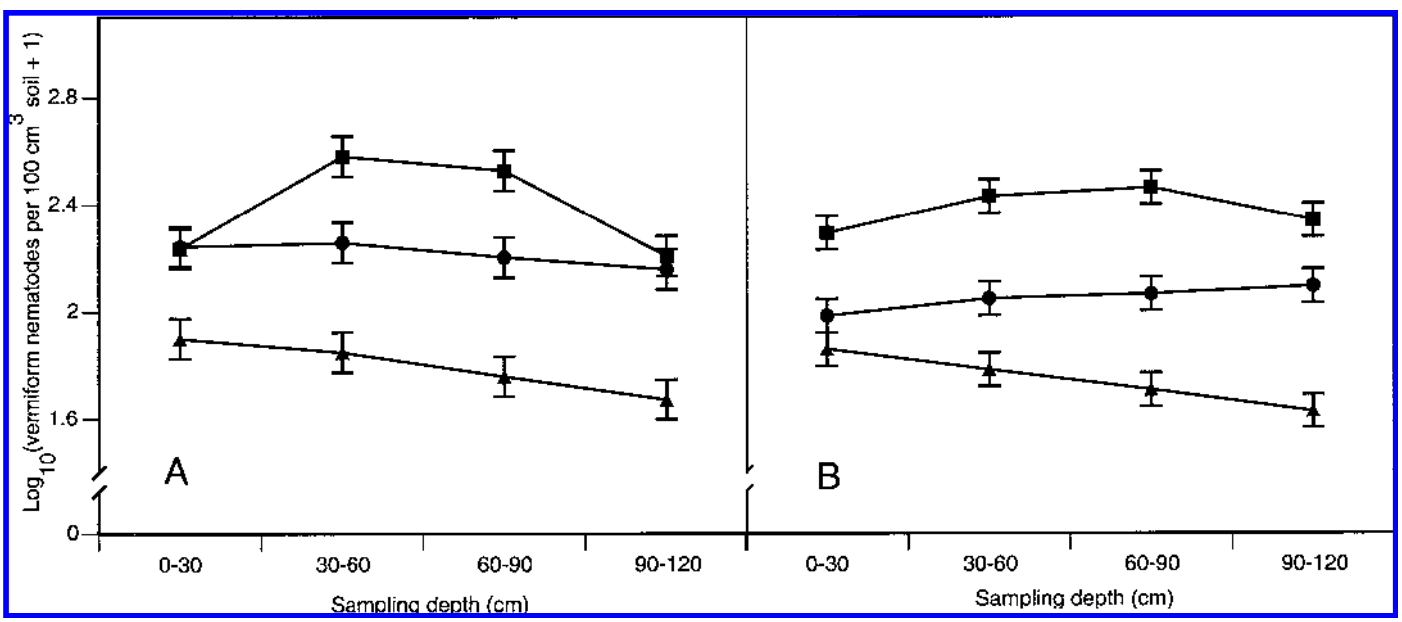

Fig. 2. Population density of *Rotylenchulus reniformis* (data transformed to $\log_{10}(x + 1)$) at four sampling depths, with three different crop sequences (■ = CS1; ● = CS2; ▲ = CS3), as defined in Table 1, averaged for three tillage systems and three sampling times in (**A**) 2000 and (**B**) 2001. The error bars represent the least significant interval (LSI), where LSI = 0.5 × $LSD_{0.05}$, for comparing crop sequences within a sampling depth and year. Values are significantly different when the error bars do not overlap.

lation plus the amendment of 100 vermiform nematodes per pot constituted the initial population of *R. reniformis* (P_i). The pots were planted with cotton (cv. Fibermax 832, Aventis, Durham, NC) and arranged in a randomized complete block design with four replications in a greenhouse at 31 ± 5°C. After 10 weeks, plants were harvested, and top oven-dry weights and root fresh weights were determined. The final population density of *R. reniformis* (P_f) was determined by Baermann funnel extraction and reported as the number of nematodes per 100 cm^3 of soil.

Data analysis. Analysis of numbers of vermiform reniform nematodes per 100 cm^3 of soil and centimeters of root length per 100 cm^3 of soil was conducted after log transformation of the data ($\log_{10}(x + 1)$). Analysis of variance (ANOVA) in SAS (SAS Institute, Cary, NC) was used in a split-split plot design with tillage, crop sequence, sampling time, and sampling depth as split blocks for each year separately. For the midseason corn plots in 2000, the population density of *R. reniformis* in the deepest soil layer was estimated proportionally (sampling time and sampling depth). For analysis within CS1, the experimental design was split-plot (tillage and sampling time) split-block (sampling depth). In CS1 only, nematode numbers and soil moisture were analyzed in a combined 2-year analysis for 2000 and 2001 after the homogeneity of error variances had been confirmed. Soil moisture was a covariate in the combined analysis for 2000 and 2001. Root length was a covariate for analysis in 2001. Wherever possible, where the majority of error terms were not significant ($P = 0.25$), the error terms were pooled. The temperature sum (average daily temperature above 15°C) was calculated for one block in CS1 cotton plots in 2001, on the basis of published information (12,28). The greenhouse experiment was a two-factor randomized complete block design. Reproductive factors were calculated from $r = P_f/P_i$, and log-transformed values ($\log_{10}(x + 1)$) were used for ANOVA.

RESULTS

Field trial. A summary of the effects of tillage, crop sequence, and depth on log-transformed population densities of *Rotylenchulus reniformis* in the 2000 and 2001 field trial is given in Table 2. The results were as follows.

Crop sequence effects during the season across tillage systems and soil depths. At the beginning of the 2000 season, population densities of *R. reniformis* were greatest in CS1, less great in CS2, and lowest in CS3 (Fig. 1A). At midseason, population densities in CS2 increased to the level of CS1, while they remained low in CS3 (Fig. 1A). At harvest, population densities were at the same high level in CS1 and CS2, while they were lower in CS3 (Fig. 1A). In 2001, population densities were at the same level in CS1 and CS2 at the beginning of the season. They were lower in CS3 than in CS1 and CS2 during the entire season (Fig. 1B). Population densities increased in CS1 during the season and decreased or remained low in CS2 and CS3 (Fig. 1B). In both years, the highest population densities developed in CS1, the second highest in CS2, and the lowest in CS3 ($P < 0.01$).

Crop sequence effects on depth distribution across tillage systems and seasons. In 2000, population densities were greater in CS1 than CS2 at the 30- to 60- and 60- to 90-cm soil depths but similar at the 0- to 30- and 90- to 120-cm depths (Fig. 2A). Population densities were lower in CS3 than in CS1 and CS2 at all soil depths (Fig. 2A). In 2001, population densities were greatest in CS1, less great in CS2, and least in CS3 at all depths, with the exception of the 0- to 30-cm depth in CS2 and CS3 (Fig. 2B).

Crop sequence effects throughout the season across tillage systems in 2000. In CS1 at midseason, population densities at the 30- to 60- and 60- to 90-cm depths were greater than at preseason, whereas population densities were similar at the 0- to 30- and 90- to 120-cm depths. At all soil depths, population densities were greater at harvest than at planting and midseason (Fig. 3A). A similar pattern was observed in CS2; population densities were more uniformly greater throughout the soil profile at midseason and again at harvest than at preseason (Fig. 3B). In CS3, population densities remained low throughout the soil profile and were lower at harvest than at preseason at the 0- to 30-, 30- to 60-, and 60- to 90-cm depths (Fig. 3C).

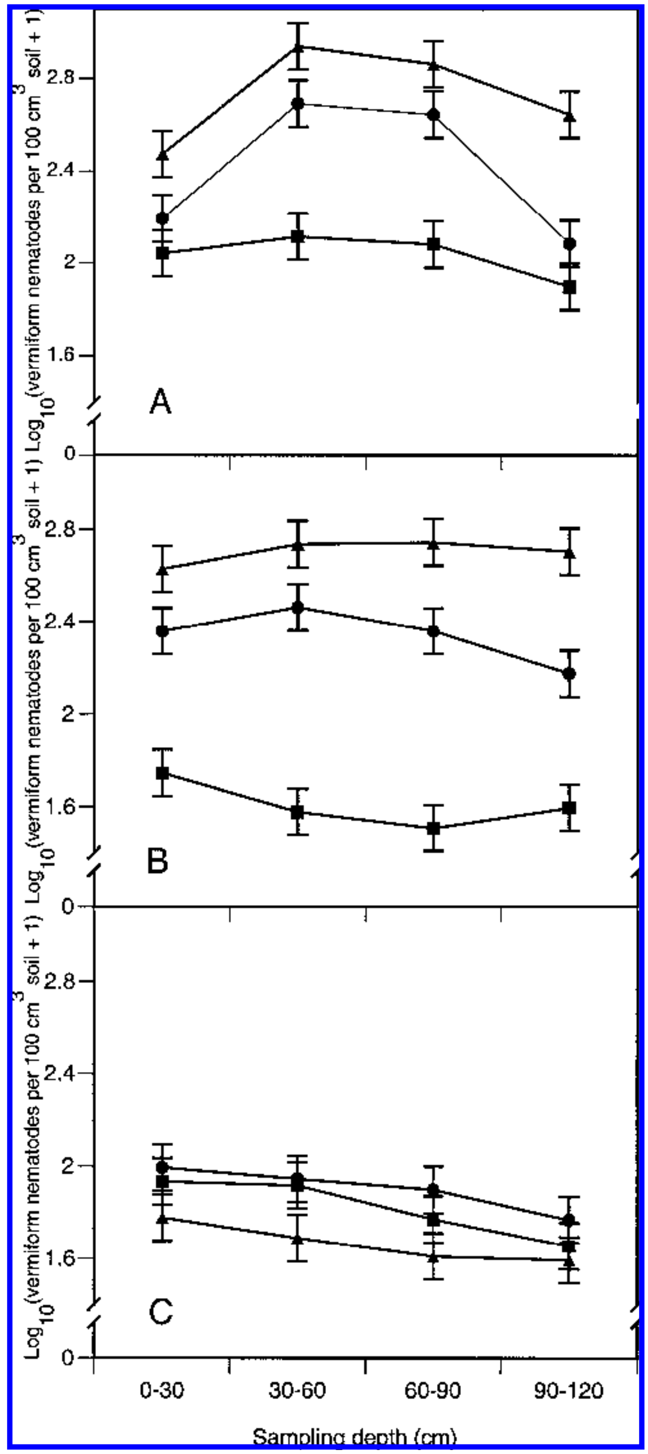

Fig. 3. Population density of *Rotylenchulus reniformis* (data transformed to $\log_{10}(x + 1)$) at four sampling depths and three sampling times (■ = preseason; ● = midseason; ▲ = harvest) for different crop sequences (CS) averaged across three different tillage systems in 2000. **A,** CS1: double-cropped with cotton followed by corn every year. **B,** CS2: double-cropped with cotton followed by corn in 2000 and planted with corn alone in other years. **C,** CS3: planted with cotton in 1999 and corn in 2000. The error bars represent the least significant interval (LSI), where LSI = 0.5 × $LSD_{0.05}$, for comparing sampling times within a crop sequence and sampling depth. Values are significantly different when the error bars do not overlap.

Additional effects in CS1 in the combined analysis for 2000 and 2001. Nematode population density data were homogeneous under cotton in CS1. Population density data were analyzed in a combined model, and the effects were summarized in an ANOVA table (Table 3).

Population densities of R. reniformis in CS1. The tillage × sampling depth and sampling time × sampling depth interactions were both significant for *R. reniformis* population densities (Table 3). Population densities at the 60- to 90- and 90- to 120-cm depths were greater in CT than in RT and NT, while population densities were similar in the three tillage systems at the 0- to 30- and 30- to 60-cm depths (Fig. 4 and Table 3). Tillage had a significant effect on population densities, with similar levels in RT and NT but greater levels in CT (Table 3).

Soil moisture in CS1. Soil moisture in 2000 and 2001 increased with soil depth, over a range from 7 to 23%. Tillage ($P = 0.0650$), the tillage × depth interaction ($P = 0.3857$), and the tillage × sampling time interaction ($P = 0.6250$) had no effect on soil moisture. There was no covariate effect of soil moisture on *R. reniformis* population density (data not shown).

Root length at midseason and harvest in 2001. Cotton root length declined with increasing soil depth at both sampling times (Table 4). Roots in the 0- to 30-cm layer were shorter at harvest than at midseason (Fig. 5). In the 30- to 60-cm layer, root lengths were similar at the two sampling times. In the 60- to 90- and 90- to 120-cm layers, they were greater at harvest than at midseason (Fig. 5). There was no covariate effect of root length on *R. reniformis* population density (data not shown).

Soil temperature effects on root length and reniform nematodes per root length. When log-transformed root length data were plotted over the corresponding temperature sum, grouping of the root lengths in differences was according to soil depth more than according to soil temperature at those depths both at midseason and at harvest (Fig. 6A). Nematode population densities per root length increased with decreasing soil depth at both sampling times (Fig. 6B), but only the depth effect was significant ($P < 0.01$).

Greenhouse test for nematode population development in soil from CS1. Plant top dry weights and root fresh weights decreased with depth origin; no tillage × depth interaction was found

TABLE 3. Analysis of variance of effects of tillage on population density of *Rotylenchulus reniformis* under cotton in a crop sequence of spring cotton and fall corn in 2000 and 2001[a,b]

	df	Mean square	Probability
Year (Y)	1	0.002987	0.87
Tillage (Tl)	2	0.585678	<0.01
Sampling time (St)	2	5.884033	0.15
Y × St	2	1.035547	<0.01
Pooled error (YTl + YTlSt)	6	0.023358	…
Sampling depth (Sd)	3	1.278951	0.06
Error (c)	18	0.048622	0.25
Tl × Sd	6	0.215309	0.01
St × Sd	6	0.213167	0.01
Pooled error (YTlSd + YStSd + YTlStSd)	24	0.061848	…
Y × Tl × Sd	(6)	(0.033285)	0.54
Y × St × Sd	(6)	(0.123880)	<0.01
Y × Tl × St × Sd	(12)	(0.045113)	0.34
Pooled error (d + e)	144	0.039785	…

[a] Only main effects and interactions discussed in the text and the corresponding error terms are shown. Data for 2000 and 2001 were combined.
[b] Population density data were transformed to $\log_{10}$[(vermiform nematodes)/(100 cm^3) + 1].

TABLE 4. Analysis of variance (ANOVA) of effects on cotton root length in a crop sequence of spring cotton and fall corn every year in a long-term field test[a,b]

	df	Mean square	*F* value	Probability
Tillage (Tl)	2	0.085385	1.28	0.31
Sampling time (St)	1	0.199417	2.99	0.10
Pooled error (a + b)	15	0.066594	…	…
Sampling depth (Sd)	3	3.516319	165.90	<0.01
Error (c)	9	0.021196	0.88	0.55
Tl × Sd	6	0.010745	0.45	0.84
St × Sd	6	0.648012	26.95	<0.01
Pooled error (d + e)	45	0.024049	…	…

[a] Cotton root lengths were measured in 2001. Only main effects and interactions discussed in the text and the corresponding error terms are listed in this abbreviated ANOVA table.
[b] Cotton root length data were transformed to $\log_{10}$[(root length, in cm)/100 cm^3) + 1].

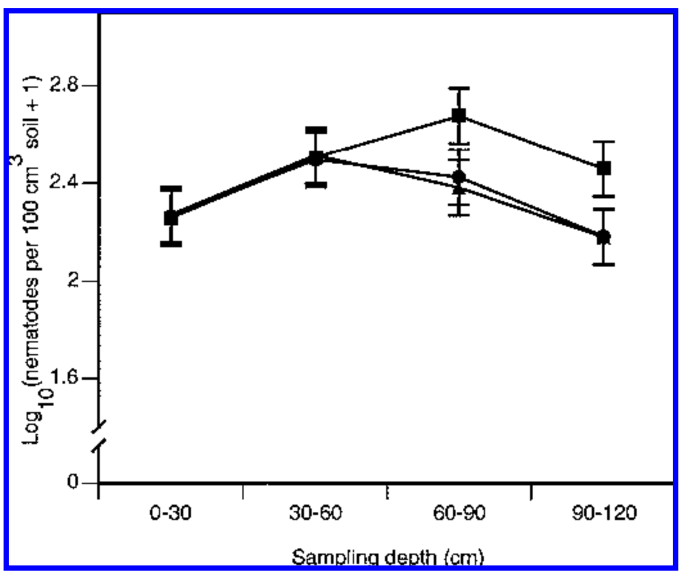

Fig. 4. Population density of *Rotylenchulus reniformis* (data transformed to $\log_{10}(x + 1)$) across the growing season in 2000 and 2001 at four sampling depths in plots double-cropped with cotton followed by corn every year (CS1), in three different tillage systems (■ = conventional tillage; ● = reduced tillage; ▲ = no-tillage). The error bars represent the least significant interval (LSI), where LSI = 0.5 × $LSD_{0.05}$, for comparing tillage systems within a sampling depth. Values are significantly different when the error bars do not overlap.

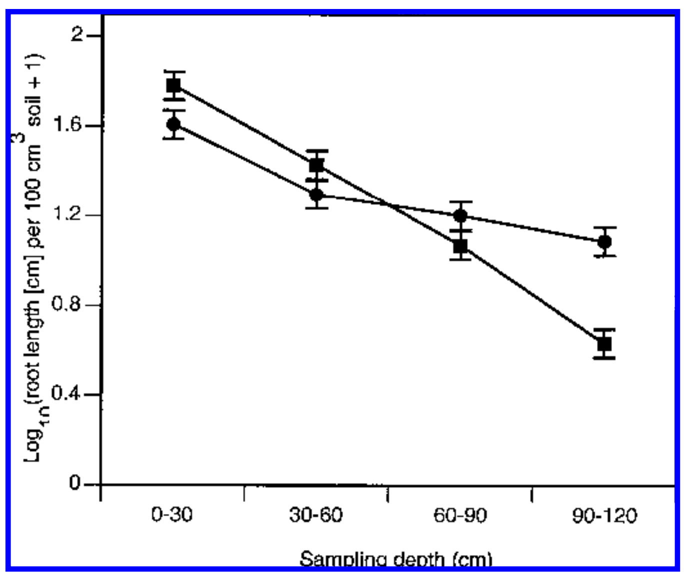

Fig. 5. Cotton root length (data transformed to $\log_{10}(x + 1)$) at four sampling depths in plots double-cropped with cotton followed by corn every year (CS1) across three different tillage systems at midseason (■) and at harvest (●) in 2001. The error bars represent the least significant interval (LSI), where LSI = 0.5 × $LSD_{0.05}$, for comparing sampling times within a sampling depth. Values are significantly different when the error bars do not overlap.

(Table 5). At harvest, final population densities of *R. reniformis* were greater in soil derived from the 60- to 90- and 90- to 120-cm layers than in soil derived from the 0- to 30- and 30- to 60-cm layers; no tillage × depth interaction was found (Table 5). For the nematode reproductive factor, the tillage × depth interaction was significant ($P = 0.03$). Soil derived from NT and RT plots permitted higher reproductive factors than soil from CT plots (Fig. 7).

DISCUSSION

Rotylenchulus reniformis was found at high population densities at depths of 30 to 60, 60 to 90, and 90 to 120 cm, confirming recent reports that populations of the nematode are present deep in the soil (29,30) but contradicting older information that considered deep-occurring populations solely a curiosity (9). Soil sampled for plant-parasitic nematodes is not usually collected at such depths. Some studies have focused on the depth distribution of *Xiphinema* spp. and *Longidorus* spp. (8,34). In California, the likelihood of detecting *L. africanus* was greatest at depths of 60 to 90 cm, but overall population densities were similar at different depths (27). In England, *X. diversicaudatum* and *X. vuittenezi* preferred soil depths of 0 to 50 cm, while *L. macrosoma* and *L. profundorum* preferred depths of 50 to 100 cm (8). In a previous study including smaller nematode species, *Trichodorus pachydermus, T. sparsus,* and *Longidorus* spp. preferred soil depths of less than 75 cm in an artificial soil profile generated in PVC tubes packed with similar soil for all depths of investigation (34). In a study of vertical distribution in an undisturbed soil profile, vertical distributions of three nematodes were associated with the soil texture preferences of the three species, resulting in greater population densities in the depth with the preferred soil texture (4). The population density of *Pratylenchus brachyurus* was greatest at depths of 45 to 90 cm, whereas that of *Belonolaimus longicaudatus* and that of *Paratrichodorus christiei* were greatest at depths of 0 to 45 cm (4). While high percentages of silt were indicative of regional distribution of high population densities of *R. reniformis*

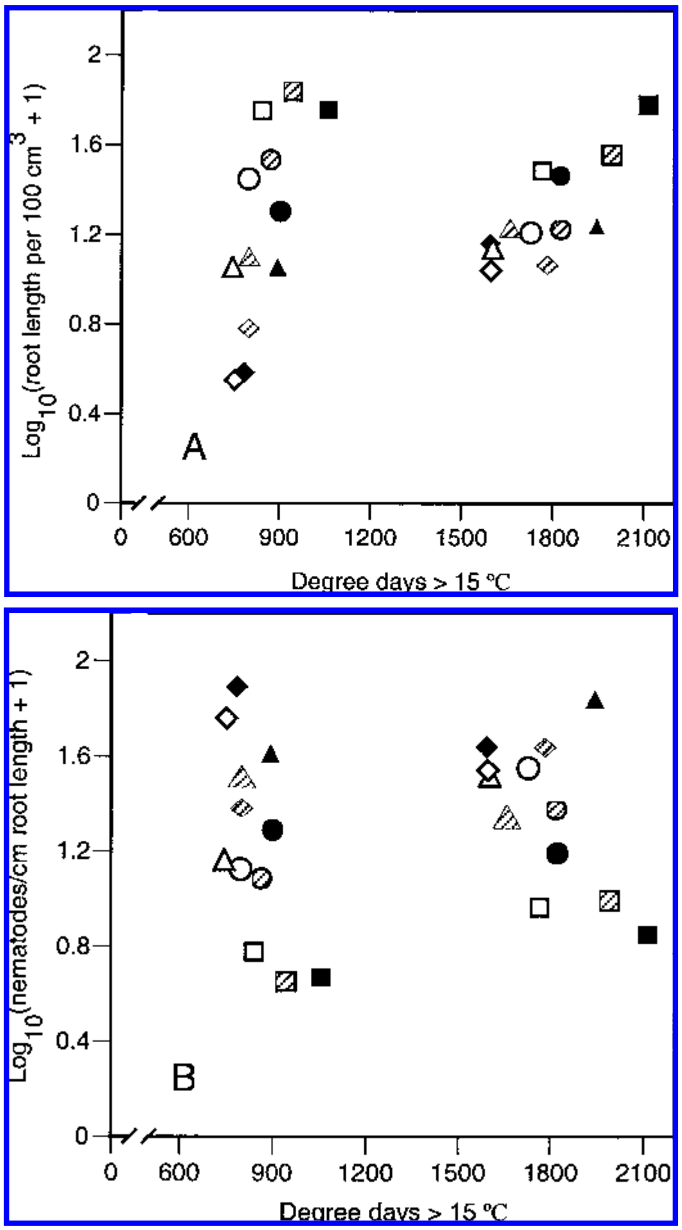

Fig. 6. Scattergrams of **A,** cotton root length and **B,** nematode population density per centimeter of root length versus degree-days in plots double-cropped with cotton followed by corn every year (CS1) in three different tillage systems, at midseason (600 to 1,200 degree-days) and at harvest (1,500 to 2,100 degree-days) in 2001. Root length data were transformed to $\log_{10}$(length + 1). Nematode population density per root length data were transformed to $\log_{10}$[(nematode count)/(root length) + 1]. Sampling depths are indicated by shape: ■ = 0 to 30 cm; ● = 30 to 60 cm; ▲ = 60 to 90 cm; ◆ = 90 to 120 cm. Tillage treatments are indicated by the fill pattern: conventional tillage is solid black; reduced tillage is cross-hatched; no-tillage is white.

TABLE 5. Top dry weight, root fresh weight, and final population density of *Rotylenchulus reniformis* under cotton in soil derived from a long-term field test

Soil depth (cm)	Shoot dry weight (g)	Root fresh weight (g)	Nematodes per 100 cm³ soil at harvest[a]
0–30	5.91	3.74	2.78
30–60	5.29	3.17	2.74
60–90	4.99	2.98	3.32
90–120	4.65	2.90	3.48
LSD—depth	0.85	0.56	0.22
P depth (D)	0.04	0.02	<0.01

[a] Population density data were transformed to $\log_{10}$[(nematode count)/(100 cm^3) + 1].

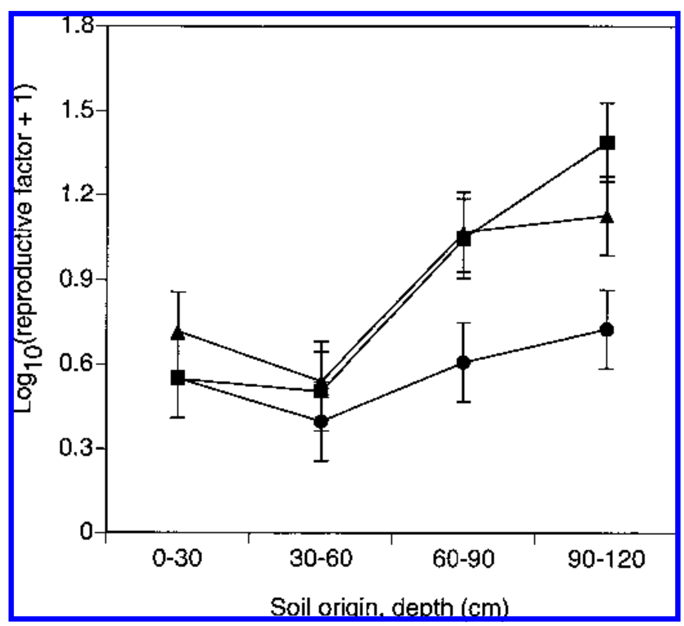

Fig. 7. Reproductive factor of *Rotylenchulus reniformis* (data transformed to $\log_{10}(P_f/P_i + 1)$, where P_i is the initial nematode population and P_f is the final population) in greenhouse tests, in soil collected from plots maintained with conventional tillage (●), reduced tillage (▲), and no-tillage (■). The sampling depth × tillage interaction was significant ($P = 0.03$). The error bars represent the least significant interval (LSI), where LSI = 0.5 × $LSD_{0.05}$. Values are significantly different when the error bars do not overlap.

(32), vertical changes in soil texture in the current study were limited and assumed not be critical in affecting changes in population density. In the study of community structure under barley and grass, strong crop impacts on nematode distribution were detected, but only 45 cm deep (42). Crop sequence and tillage systems did affect reniform nematode population densities at depths of 30 to 120 cm. Differences in population densities were not associated with soil moisture. Cotton root length and the corresponding availability of nematode feeding sites appeared not to affect nematode population densities. As soil depth increased, root length decreased, but more reniform nematodes were found.

In 2000 and 2001, crop sequence effects were in agreement with the common hypothesis that nonhost crops reduce nematode population densities (1). Crop sequences in which susceptible host plants alternate with nonhosts are commonly used to keep plant-parasitic nematode population densities below damage threshold levels in low-value crops (5). In our test, effects of crop sequence on nematode population densities were measurable to a depth of at least 120 cm. In both 2000 and 2001, population densities were highest in the plots double-cropped with cotton and corn (CS1). In 2000 (double cropping in CS2), nematode population densities under cotton in CS2 increased to the same level as in CS1 during the season. At the preseason sampling, the lowest population densities were present in CS2 plots after 2 years of corn, but nematode reproduction was not obviously limited in CS2 cotton in 2000. A second year of a nonhost crop (grain sorghum in CS3 in 2001) resulted in limited additional reduction of population densities, after the nematode population had been reduced by 1 year of corn (2000 in CS3 and 2001 in CS2).

In CS1, *R. reniformis* may have reached its optimum population density for the ecological niche in this field. In CS2, the "ceiling" for nematode population density was reached within one season, as has been described for many plant-parasitic nematodes. This observation demonstrated the benefit of incorporating nonhost crops into crop sequences: the nematode pest is reduced under a nonhost crop, but high population densities develop under a susceptible host in the crop sequence. The rapid population increase in the year in which the host crop is planted makes it necessary to rotate out of the host after one cropping season. Plant-parasitic nematodes interact with other soilborne organisms, including antagonists and soilborne pathogens (15,16,37). Limited attention has been given to the effect of crop sequences and antagonistic interactions. In current crop sequences, possible natural population density reduction is considered less important than direct reduction of population densities under nonhosts. In a study of a *Heterodera schachtii*–suppressive soil, suppressiveness was lost after two crops of the nonhost wheat (49). When crop sequences are being designed to keep nematode population densities low throughout the sequence, the rapid reestablishment of population densities under a host crop following a nonhost should be considered. Possibly natural population regulation needs more consideration in the design of crop sequences.

In the combined analysis in the intensive crop sequence CS1, nematode population densities were higher in the CT plots than in the RT and NT plots at soil depths of 60 to 90 and 90 to 120 cm, where direct tillage effects were not expected. Soil moisture increased with soil depth, and root length decreased with depth. Both parameters had no measurable covariate effect on nematode population density. Overall, fewer cotton roots were present at greater depths, and possibly nematode population development at these depths was limited by the availability of fewer feeding sites. At harvest, when more roots than at midseason were available in deeper soil layers, nematode population densities increased the most. When soil was removed from the field and planted with cotton in the greenhouse, differences in final reniform nematode population densities continued to be associated with the depth at which the soil originated, even though root densities were similar (1.3-fold versus ninefold, from the shallowest to the deepest). We hypothesized that an undefined factor present only at shallower depths in the soil reduced nematode population density. Soils from NT and RT plots permitted higher reproduction at greater depths than soils from CT plots, suggesting that tillage and the physical location in the soil profile are important in restricting nematode reproduction. In long-term tillage studies of soybean cyst nematodes, it was proposed that the increased activity of nematode pathogens and parasites may be responsible for lower cyst nematode population densities under no-tillage than rigorous tillage (47). The important role of high soil moisture in nematode suppression is apparent in the density regulation of *H. avenae,* which is suppressed in northern Europe in moist but not dry years (17). It is well established that soil microbial populations exhibit suppressive effects on nematode population densities (16,37,48). Such organisms may have been present in these soils.

The impact of no-tillage on the distribution of *R. reniformis* is probably less important than its effects on other factors, such as soil fertility. The impact of deep-occurring reniform nematode populations on plant growth has been under investigation in parallel studies (30,31; Westphal et al., *unpublished*). This project demonstrated substantial effects of tillage practices and crop sequence on the entire agroecosystem in population densities of the plant-parasitic *R. reniformis.*

ACKNOWLEDGMENTS

This paper is published with the Agricultural Research Program, Purdue University, approval number 16947. We thank the Department of Plant Pathology and Microbiology of Texas A&M University; the Kika de la Garza Subtropical Agricultural Research Center of the U.S. Department of Agriculture, Agricultural Research Service, in particular the Integrated Farming and Natural Resources Unit, under the direction of J. Bradford; the Texas Agricultural Experiment Station; and the Texas Cooperative Extension for support. We thank C. Adams and J. Santini for discussion of statistical procedures; L. Dunkle, A. F. Robinson, J. Starr, and B. Wiedenfeld for valuable discussion and support; and J. Alejandro, J. Brockington, J. Campos, and V. Valladares for technical assistance. We thank the Department of Botany and Plant Pathology and the Department of Agronomy at Purdue University for their support.

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